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杂色鲍幼虫附着变态相关功能基因的研究

Studies on functional genes involved in larval settlement and metamorphosis in the small abalone *Haliotis diversicolor*

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摘要

杂色鲍 (*Haliotis diversicolor*) 是我国南方水产养殖的重要养殖种类。附着变态是杂色鲍早期发育过程的关键时期, 幼虫敏感而脆弱, 其变态成功与否将影响到种群分布和数量变动。因此研究杂色鲍幼虫附着变态相关的功能基因对于深入揭示幼虫附着变态分子机制具有重要理论意义, 并为生产实践提供指导。

本文从实验室构建的杂色鲍转录组数据库中, 克隆获得了与 GABA 相关的四个基因的序列片段: glutamic acid decarboxylase、GABAA Receptor ζ subunit、GABAB Receptor $\alpha 1$ subunit and GABAA Receptor ρ subunit, 分别命名为 HdGAD、HdGABA_A- ζ 、HdGABA_B- $\alpha 1$ 、HdGABA_A- ρ , 并对这 4 个基因在杂色鲍幼虫附着变态过程中的作用进行研究, 主要结果如下:

1) 杂色鲍 HdGAD 基因的 cDNA 序列全长 2331bp, 开放阅读框 (ORF) 长 1710bp, 编码 569 个氨基酸, 预测蛋白含有一个特殊的 DOPA_deC_like 功能域, 一个 GadA 功能域, 有一个 CSdA 多功能域, 属于 Pyridoxal phosphate-dependent decarboxylase (IPR002129) 家族。HdGAD 基因组 DNA 全长为 4908bp, 包含 4 个外显子和 3 个内含子。Real-time PCR 结果显示, HdGAD 在杂色鲍早期胚胎发育各个时期均有表达, 自面盘幼体中期, 其表达水平急剧上升, 且在匍匐幼体早期和匍匐幼体中期维持较高的表达水平。RNAi 技术沉默 HdGAD 发现, 下调后的 HdGAD 对杂色鲍幼虫的附着变态影响效果不明显 ($P>0.05$), 而且不会影响杂色鲍幼虫的生存 ($P>0.05$)。

2) 杂色鲍 HdGABA_A- ζ 基因的 5' 端序列长 1362bp, 部分开放阅读框 (ORF) 长 1069bp, 可编码 356 个氨基酸, 含有 1 个 Neur_chan_lig-bd 结构域, 1 个神经递质配体门控离子通道跨膜结构域, 属于 gama-aminobutric acid A receptor/Glycine receptor alpha 家族。Real-time PCR 结果显示, HdGABA_A- ζ 基因在杂色鲍胚胎发育的各时期表达量有着明显不同: 从卵、受精卵到桑葚胚之前的时期, HdGABA_A- ζ 基因的表达水平很低, 且在受精卵和多细胞时期检测不到信号 ($C_t \geq 35$); 而从面盘时期开始到匍匐中期, HdGABA_A- ζ 的表达量维持

较高水平。RNAi 技术沉默 HdGABA_A- ζ 来研究其对杂色鲍附着变态中的作用发现, 下调后的 HdGABA_A- ζ 能明显使杂色鲍幼虫的附着率降低 ($P<0.05$), 死亡率升高 ($P<0.05$)。

3) 杂色鲍 HdGABA_B- $\alpha 1$ 基因的 5'端序列长 1890bp, 部分开放阅读框(ORF) 长 1861bp, 可编码 620 个氨基酸, 含有 1 个 PBP1_GABA_b_receptor 结构域, 一个 7tm_3 结构域, 一个 LivK 结构域, 一个 ANF_receptor 结构域, 属于 Periplasmic_binding_protein_Type_1 和 7tm_3 超家族。Real-time PCR 结果显示, HdGABA_B- $\alpha 1$ 基因在杂色鲍胚胎发育的各时期都有较高水平的表达, 随着各时期的变化而有所不同, 且在匍匐早期表达水平最高, 是卵子表达量的 34.6 倍。RNAi 技术沉默 HdGABA_B- $\alpha 1$ 来研究其对杂色鲍附着变态中的作用发现, 沉默后的 HdGABA_B- $\alpha 1$ 对杂色鲍幼虫的附着变态和生存无明显影响 ($P>0.05$)。

4) 杂色鲍 HdGABA_A- ρ 基因的 cDNA 序列全长 2338bp, 开放阅读框(ORF) 长 1038bp, 可编码 345 个氨基酸, 含有一个神经递质门控通道结构域, 一个神经递质门控离子通道跨膜结构域, 一个神经递质门控离子通道配体结合域, 属于 Neurotransmitter-gated ion-channel (IPR006201) 家族。Real-time PCR 结果显示, HdGABA_A- ρ 基因在杂色鲍胚胎发育的早期表达水平很低, 表达信号很弱 ($C_t \geq 35$), 而 HdGABA_A- ρ 从匍匐早期开始明显地表达, 其后的匍匐中期也维持较高水平的表达量。RNAi 技术沉默 HdGABA_A- ρ 来研究其对杂色鲍附着变态中的作用发现, 下调后的 HdGABA_A- ρ 能极明显降低杂色鲍幼虫的附着率 ($P<0.01$), 使死亡率升高 ($P<0.05$)。

5) GABA 受体特异性激动剂的药理学实验发现, 在一定浓度范围 ($0.01-10\mu\text{mol.L}^{-1}$) 内, 蝇蕁醇 (Muscimol) 作为 GABA_A 受体的特异性激动剂能明显的促进杂色鲍幼虫附着, 而对杂色鲍幼虫的生存不会产生影响, 除了 $0.10\mu\text{mol.L}^{-1}$ 蝇蕁醇外 ($P<0.05$); 氯苯氨丁酸 (Bacofen) 作为 GABA_B 受体的特异性激动剂, 在一定范围内能促进杂色鲍幼虫的附着, 但高于 $10\mu\text{mol.L}^{-1}$, 就会影响其生存 ($P<0.01$)。

关键词: 杂色鲍; GABA 受体; 附着变态

Abstract

The abalone *Haliotis diversicolor* is one of important molluscs for aquaculture in China southern sea area. Larval settlement and metamorphosis is a key stage in the early embryo development of *Haliotis diversicolor*, and effects its population distribution and dynamics of its population, which is on the account of the sensitive and fragile condition. Thus, Studies on functional genes involved in larval settlement and metamorphosis in the small abalone *Haliotis diversicolor* may provide an important theoretical significance and guidance for *haliotis diversicolor* aquaculture.

Partial sequence of glutamic acid decarboxylase、GABAA Receptor ζ subunit、GABAB Receptor $\alpha 1$ subunit and GABAA Receptor ρ subunit ,which were related to GABA, were obtained from our EST database of *Haliotis diversicolor*, denoted as HdGAD、HdGABA_A- ζ 、HdGABA_B- $\alpha 1$ 、HdGABA_A- ρ , respectively. This study main focus on revealing the role of HdGAD、HdGABA_A- ζ 、HdGABA_B- $\alpha 1$ 、HdGABA_A- ρ in larval settlement and metamorphosis of *Haliotis diversicolor*. The main results of this study as follows:

1)The full-length cDNA of HdGAD is 2331bp, with a 1710bp ORF coding 569 aa, and the predicted protein contains a DOPA_deC_like domain、a GadA domain、a CSdA multifunctional domain, is a part of Pyridoxal phosphate-dependent decarboxylase (IPR002129) family. This study cloned genomic DNA of HdGAD is 4908bp, and comprises 4 exons and 3introns. The mRNA expression of HdGAD in several small abalone larval development stages is constitutively expressed in all larval development stages, and the highest expression level was in the mid veliger stage, and the early creeping larval stage and the mid creeping larval stage had a high level as well by using Real-time PCR. RNAi was applied on larvae during the competent stage and the results show, compared with blank control, both the settlement

rate and the mortality rate of down-regulated HdGAD had no difference($P>0.05$).

2)The 5'terminal sequence of HdGABA_A- ζ is 1362bp, with a 1069bp partail ORF coding 356 aa, and the predicted protein contains an Neur_chan_lig-bd domain , an Neur_chan_transmembrane domain, is a part of gama-aminobutric acid A receptor/Glycine receptor alpha Family.The mRNA expression of HdGABA_A- ζ in several small abalone larval development stages is obviously different.The mRNA expression of HdGABA_A- ζ is very little from eggs 、 zygots to morula and even that of the zygote stage and multicellular stage can't detect the positive signal (Ct \geq 35) , but there was a high level obvious from veliger stage to the mid-creeping larval stage by using Real-time PCR. RNAi was applied on larvae during the compent stage and the result shows, compared with blank control, the settlement rate had significant drop ($P<0.05$),mean while the mortality rate of down-regulated HdGABA_A- ζ had abviously rise ($P<0.05$).

3)The 5'terminal sequence of HdGABA_B- α 1 is 1890bp, with a 1861bp partail ORF coding 620 aa, and the predicted protein contains a PBPI_GABA_b_receptor domain, a 7tm_3 domain, a LivK domain, an ANF_receptor domain, is a part of Periplasmic_binding_protein_Type_1 and 7tm_3superfamily. The mRNA expression of HdGABA_B- α 1 in several small abalone larval development stages is constitutively expressed in all larval development stages, and the highest expression level was in the early creeping stage,with 34-fold higher than that of eggs by using Real-time PCR. RNAi was applied on larvae during the compent stage and the results shows,compared with blank control,both the settlement rate and the mortality rate of down-regulated HdGABA_B- α 1 had no obviously difference($P>0.05$).

4)The full-length cDNA of HdGABA_A- ρ is 2338bp, with a 1038bp ORF coding 345 aa, and the predicted protein contains a neurotransmitter-gated ion-channel domain,a Neur_chan_lig-bd domain , a Neur_chan_transmembrane domain, is a part of neurotransmitter-gated ion-channel (IPR006201) family,which is the first time to

obtain the HdGABA_A-p of *Haliotis diversicolor* so far. The mRNA of HdGABA_A-p in several small abalone larval development stages is obviously different. The high expression level was in the early creeping larval stage and the mid-creeping larval stage by using Real-time PCR. RNAi was applied on larvae during the competent stage and the result shows, compared with blank control, the settlement rate had significantly drop ($P < 0.01$) and the mortality rate of down-regulated HdGABA_A-p had obviously rise ($P < 0.05$).

5) The results of Pharmacology experiment on antagonists of GABA receptors revealed that muscimol as the special GABA_A receptor, can promote the larval settlement rate of *Haliotis diversicolor* significantly, and make no difference on larval survival, except $0.10 \mu\text{mol.L}^{-1}$ muscimol ($P < 0.05$); Baclofen as the special GABA_A receptor, can promote the larval settlement rate of *Haliotis diversicolor* significantly, but will effect the larval survival when the concentration of baclofen out of range $10 \mu\text{mol.L}^{-1}$.

KeyWords: *Haliotis diversicolor*; GABA receptors; settlement and metamorphosis

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